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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,837	10/15/2003	Jost Seibler	100725-38 / Kreisler 1109	8812

27384 7590 05/17/2007

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ART UNIT	**PAPER NUMBER**
1632	

MAIL DATE	DELIVERY MODE
05/17/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/685,837	SEIBLER ET AL.	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 January 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5,6,8-24,26,27,29 and 30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 5-6, 8-24, 26-27 and 29-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on January 26, 2007, has been received and entered. Claims 1, 8-9, 2, 29 and 30 have been amended and claims 2-4, 7, 25 and 28 have been canceled.

Claims 1, 5-6, 8-24, 26-27, 29 and 30 are pending in the instant application.

Election/Restrictions

Applicant's election with traverse of the invention of group IV (27) filed October 24, 2005 was acknowledged. Applicant argument of examining method for gene knock down in a vertebrate (group 1) with elected group were found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate were rejoined for the examination purposes.

Accordingly, a method for gene knock down in a vertebrate and vertebrate having stable integration at Polymerase II dependent locus, an expression vector comprising an shRNA construct under control of a ubiquitous promoter will be examined in the instant application.

Claims 1, 5-6, 8-24, 26-27, 29 and 30 are under consideration.

Withdrawn-Claim Objections

The objection to claims 1-3, 5-24, 26-27 and 29-30 is withdrawn in view of amendments to the claims now rewritten in independent form to recite elected invention.

Withdrawn-Claim Objections

The objection to claim 2 as being of improper dependent form for failing to further limit the subject matter of a previous claim is withdrawn in view of cancellation of claim 2.

New-Claim Objections

Claim15-17 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. In the instant case, claim 1 is directed to a method of gene knock down in a nonhuman vertebrate by stably integrating an expression vector comprising shRNA by homologous recombination at polymerase II dependent locus of the genome, while dependent claims 15-17 do not further limit claim 1 rather they limit the vector to be integrated to an Polymerase III dependent locus. Appropriate correction is required.

Withdrawn- Claim Rejection- 35 USC § 112

Claim 29 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of amendments to claim 29.

Maintained- Necessitated by amendments-Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 5-6, 8-24, 26-27, 29 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of gene knockdown in a mouse genome at the *rosa26* locus, said method comprising introducing a reporter construct comprising shRNA in mouse embryonic stem cell by homologous recombination, wherein said shRNA and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice acceptor sequence

and polyadenlylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter and terminated by five thymidines; and microinjecting said mouse embryonic stem cell into mouse diploid blastocysts; and implanting the blastocysts comprising the mouse embryonic stem cell into pseudo pregnant mouse; allowing the resulting pregnant mouse to deliver viable chimeric offspring and a transgenic mouse produced by said method, wherein said transgenic mouse exhibits ~90% reduced luciferase activity in liver, heart, brain and muscle, does not reasonably provide enablement for the method of gene knockdown in any other nonhuman vertebrate using any other promoter with any other shRNA sequence. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claim 1 encompasses a method for constitutive and/or inducible gene knock down in a nonhuman vertebrate comprising an shRNA construct under control of a ubiquitous promoter and homologous sequence which integrates through homologous recombination at the polymerase II dependent locus of the genome of the nonhuman vertebrate. The dependent claim 5 limits the polymerase II dependent locus selected from list of a group consisting of rosa26, collagen, RNA polymerase, actin and HPRT locus. Claims 6, 8-14 encompasses the expression vector of claim 1, which further contains functional sequences and group of promoters, which is either constitutive or inducible. The inducible promoter expression is a promoter consisting an operator sequence and the vertebrate of claim 1 is non-human vertebrate, which is further limited to either mouse or fish. Claims 15-19 encompass method of claim 1 wherein the vector is a either Pol III or Pol II dependent promoter driven shRNA construct suitable for integration into a Pol II dependent locus. Subsequent claim limit the Pol III promoter being either constitutive or inducible H1 or U6 promoter while Pol II promoter being inducible CMV promoter. Claims 20-24, 26-27 encompasses method of claim 1 which describes the shRNA segment comprising a stop and or polyadenylation sequence that is integrated at polymerase dependent locus of the vertebrate and ES cell of the vertebrate.

The application as filed is not enabling for the invention commensurate with the full scope of the claims because art of gene knockdown by shRNA stably integrating by homologous recombination into a polymerase Ii dependent locus by any method is unpredictable as has been recognized by the art of skill and therefore require undue experimentation. As will be shown below, these broad aspects as well as limitations were not enabled for the claimed invention commensurate with the full scope of the claims at the time of filling of this application because neither the specification nor the art of record taught sufficient guidance to practice the claimed invention commensurate with the scope of the claim.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

The office has analyzed the specification in direct accordance to the factors outlines in *In re Wands*. MPEP 2164.04 states: "[W]hile the analysis and conclusion of a lack of enablement are based on factors discussed in MPEP 2164.01(a) and the evidence as whole, it is not necessary to discuss each factor in written enablement rejection." These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled.

Claims 1, 5-6, 8-24, 26-27 and 29 are broad in scope. The following paragraph will outline the full scope of the claims: Claimed invention recites a method of gene knockdown in a nonhuman vertebrate, wherein said animal comprises stably integrated by homologous recombination at polymerase II dependent locus of the any nonhuman

vertebrate, an expression vector comprising a short hairpin RNA (shRNA) constructs under control of any ubiquitous promoter. Since these claims are broad in scope, encompassing any nonhuman vertebrate having stably integrated by homologous recombination at Pol II dependent locus by any method using any promoter, subsequently limiting to few ubiquitous promoter, the disclosure provided by the applicant, in view of prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of those, aspect considered broad must be shown to a reasonable extent so that one of the ordinary skills in the art at the time of invention by applicant would be able to practice the invention without any undue burden being on such Artisan.

The specification broadly discloses the progression of RNA interference technology over the years (pp. 1,2) and describes role of shRNA-mediated gene silencing in transgenic mice and rats. The invention is based in part of a method of using a expression vector comprising a short hairpin RNA construct under the control of ubiquitous promoter for gene knock down in a living organism (pp 3). Pages 4-6 provide short description of figures. Pages 7-14 of the specification disclose definition of terms, general description of ubiquitous promoter, expression vector and a general description of different shRNA sequence in tabular form. Pages 15-17 broadly discusses preferred embodiments of the method steps comprising of generating shRNA and construct for cell culture, luciferase measurement assay and generation of chimeric mice. Example 1: of specification teaches the firefly luciferase gene along with a splice acceptor sequence is inserted into first allele of rosa26 locus by homologous recombination in ES cells while shRNA and Renilla luciferase gene is inserted into second allele of rosa 26. Figure 7 shows the expression of the firefly luciferase in presence and absence of shRNA expression cassette. Example 2 shows shRNA expression cassette under control of U6 promoter containing tet operator sequence and a Renilla luciferase gene is inserted into first allele of rosa26 locus (figure 8 and 12), while the luciferase gene with a promoter and a tet repressor expression cassette is introduced into the second allele in ES cells. Luciferece activity is shown in presence and absence of doxycycline. Example 3 page 19 describes that NIH3T3 cells are transiently transfected with

construct expressing the luciferase and tet repressor gene together with the shRNA construct containing tet operator sequence. Figure 11 shows the expression of luciferase in presence and absence of doxycycline. The specification discloses doxycycline inducible shRNA expression resulted ~80% inhibition in firefly luciferase activity in cells. Example 4 pages 19 show chimeric mice from rosa26/U6 and H1-ShRNA transgene. The data shows shRNA construct under the control of both U6 and H1 effectively repressed the firefly luciferase activity inmost organs (Figure 13B).

However, such broad disclosure does not demonstrate the information required by the Artisan to reasonably make and use any nonhuman vertebrate with an expression vector comprising any short hairpin RNA (shRNA) construct under control of a any ubiquitous promoter integrated by homologous recombination at polymerase II locus by any method.

As a first issue, claim 1 embraces a method for gene knockdown in a nonhuman vertebrate comprising stably integrated an expression vector comprising a shRNA construct under control of any ubiquitous promote selected from group consisting of polymerase I-III dependent promoter into a polymerase II dependent locus of the genome of the non human vertebrate. The specification contemplated that the expression vector of the instant invention is suitable for stable integration into the nonhuman vertebrate. It is noted that vectors for transient integration and vector that contains homologous sequences suitable for targeted integration at a polymerase II dependent locus of the nonhuman vertebrate are also contemplated (see paragraph 32). The specification provides working example showing a method of gene knockdown by introducing a shRNA and reporter constructs in mouse embryonic stem cell by homologous recombination, wherein said shRNA and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice acceptor sequence and polyadenlylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter (see example 1 and 4).

As recited claims 1, 26 and 27 are broad and embrace integration of shRNA construct at polymerase II dependent locus of the genome of the animal by any method.

Prior to instant invention, shRNA mediated RNAi in nonhuman vertebrate especially in mice had been shown by a number of independent group using random transgnesis by pronuclear injection and transfection of shRNA construct. It is noted that each of these method resulted in aberrant pattern of shRNA expression depending on the site of transgene integration. In addition, gene silencing in these animals varied from undetectable to greater than 90% level (Tiscornia G, Proc Natl Acad Sci U S A. 2003; 100(4): 1844-8; Hasuwa et al FEBS Lett. 2002; 532(1-2): 227-30, IDS). Furthermore, these methods also failed to show any distinct phenotype (Carmell et al., Nat Struct Biol. 2003; 10(2): 91-92). Thus, it is clear from the cited art that, the resulting phenotype of a gene knockdown resulting from any method routinely used for RNAi effect for transgenic mice was considered unpredictable. In the instant case, Applicant's examples only describe a method to knock down expression of luciferase using shRNA construct using mouse ES cell and locus specific targeting of the construct by integrating vector in mouse ES cell. It is noted that none of the examples demonstrate specific *in vivo* gene silencing by any other method that correlate to stable integration of shRNA construct at polymerase II dependent locus leading to effective gene silencing. It is evident from the cited arts that without any specific guidance of how any method that integrates randomly at polymerase II dependent locus would result in sustained reproducible expression of shRNA resulting in gene silencing. Because of the art, as shown above, does not disclose how standard transgenesis by random integration of an expression construct comprising shRNA to Polymerase II dependent locus in the genome would not result in effective gene silencing. An artisan would have to carry out extensive experimentation to make use the invention, and such experimentation would have been undue because of the art of gene silencing using shRNA construct *in vivo* is unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced.

As a second issue, Claims 1, 5-6, 8-24, 27 and 29 embrace a nonhuman vertebrate and a method for constitutive gene knockdown in a nonhuman vertebrate by stably integrating by homologous recombination an expression vector into a polymerase II dependent locus of the genome of the nonhuman vertebrate. The specification has

exemplified a method that embraces an expression vector comprising shRNA for stable, locus dependent integration of the construct using embryonic stem cell. The art at the time of filing further held that transgenic technology was not predictable for any species other than mouse. Since the specification discloses using mouse ES cells to produce transgenic mice via homologous recombination of targeting vectors in the ES cells, ES cells from various species are required to produce various nonhuman vertebrates. However, Houdebine, 1994 (Journal of Biotechnology, Vol., 34, pp 269-287) describes that although ES cells can be used to generate transgenic animals, but this approach remains restricted to mice, ES cells from other species are not presently available (pp 279). Furthermore Mullin et al also point that non-mouse ES cell capable of providing germ line chimeras were not available (Mullins et al., Journal of Clinical Investigation, 1996, pp 1557, 1st paragraph). Campbell and Wilmut (1997, Therigenology, art of record) acknowledges report of ES-like cells in number of species, but also emphasize that there are no report of any cell line that contribute to germ line in any species other than mouse (pp 65; 2nd paragraph). Thus, the state of the art is such that ES cell technology is generally limited to the mouse system and that only putative ES cells exist for other species (Moreadith et al., J. Mol. Med., 1997 p214, abstract, art of record). Therefore, at the time of filing of this application, method of gene knockdown in any vertebrate could not be accomplished for any species other than mouse. The specification does not teach how to make knock down nonhuman vertebrate by shRNA for any other species other than mice or correlate making mice to making knockout for any other species. Therefore, the claims should be limited to mouse and method for gene knockdown in mouse as discussed in the office action.

As a third issue, claims 27 and 29 recites a nonhuman vertebrate with an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter integrated at a polymerase II dependent locus. However, the specification as filed does not provide any specific information about resulting phenotype of the claimed invention. It is noted that the specification merely recites the luciferase activity in different organ, however it dose not provide any specific information for practicing the claimed invention commensurate with the full scope of the claim. The

specification teaches method of making a transgenic mice-using shRNA. However, it is noted that art recognizes that the resultant phenotype, when producing knockdown mice, is exceedingly unpredictable. For example, Leonard (Immunological Reviews, 1995, 148: 98-114) discloses mice with disruption in the gc gene that was intended to be a model for X-linked severe combined immunodeficiency (XCIDS), but displays a variety of unexpected traits (Abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (pp 105, line 7). Similarly, Carmell et al failed to produce any distinct phenotype, while shRNA, constructs directed against seven known targets were introduced via standard trasngenesis (Carmell MA Nat Struct Biol. 2003; 10(2): 91-92). Thus it is clear from the cited arts that at the time of filing, the resulting phenotype of a gene knockdown resulting from methods routinely used for integrating shRNA in the genome of nonhuman vertebrate was considered unpredictable.

As a final issue, claims are directed to shRNA under the control of ubiquitous promoter and further describes expression vector is also suitable for transient integration (pp7; 4th paragraph). The unpredictability of attenuating /inhibiting expression of a target gene in cell by shRNA is evident in prior and post filing art. While it is recognized, that introduction of shRNA that is targeted to a specific gene may result in attenuation /inhibition of the targeted gene, the degree of attenuation and length of the time attenuation is achieved in not predictable (Caplen et al Gene 2000, vol. 252, 95-105, art of record). In addition, Prawitt et al. describe a recent study showing expression of shRNA in mammalian cell induced target gene for interferon pathways (Prawitt et al Cytogenet Genome Res. 105 (2-4), 412-421, 2004pp419, column 1, 2nd paragraph, references therein). In view of these studies, Prawitt et al stressed the importance of interpreting the RNAi effects both in tissue culture as well as in mouse. Furthermore, Prawitt et al evinces an optimistic outlook for tetracycline inducible system in generating inducible knock down mouse, but also acknowledges that the art is still unpredictable by stating "it remains to be proven if doxycycline can be used to study as graded knock down phenotype in mouse" (pp 419; 2nd column).

In view of lack of teaching or guidance provided by the specification with regard to an enabled method for gene knockdown in any vertebrate comprising a disruption in gene using any shRNA, construct comprising different constitutive or inducible promoter, and shRNA sequence and the lack of teaching or guidance provided by the specification to overcome the art recognized unpredictability of disruption of a particular gene, promoter and locus and the resulting phenotype and absence of any correlation between disruption and its phenotype, for the specific reason cited above in the office action. It would require undue experimentation for an Artisan to make and use the claimed invention and/or working examples demonstrating the same, such invention as claimed by the applicant is not enabled for the claimed inventions commensurate with the full scope of the claims.

Response to Arguments

Applicant's arguments filed January 26, 2006 have been fully considered but they are not persuasive. Applicants argue that at the time of filing of this application in 2003, a person skilled in the art was well in the position to perform gene knock-down/gene knock-in either randomly or through homologous recombination in any type of animal. Applicants also assert that Examiner concedes enablement for mice. Applicants also argue that it would be improper to limit to a particular phenotype since method of present invention is to provide a powerful expression system for shRNA in a given animal.

In response, it is noted that independent claim 1 is directed to a method of gene knockdown in a nonhuman vertebrate by stably integrating by homologous recombination an expression vector in polymerase II dependent locus in the genome of the nonhuman vertebrate. Examiner would agree with applicants assertion that prior art taught methods to perform gene knock-down/gene knock- randomly or through homologous recombination. However, it is noted that as amended claims require expression vector to be incorporated in the genome by homologous recombination. It is emphasized that prior art also taught that random transgenesis by pronuclear injection

and transfection of shRNA construct resulted in aberrant pattern of shRNA expression depending on the site of transgene integration and gene silencing varied from undetectable to greater than 90% level. This is further supported by studies of Carmell et al that failed to obtain distinct or reproducible phenotype expected for a hypomorphic allele of the targeted gene against seven known targets that were introduced via standard trasngenesis (supra; Carmell MA Nat Struct Biol. 2003; 10(2): 91-92). In fact, applicants while arguing against 102 rejection (Beach et al) cite Martin & Whitelaw, BioAssays 18. 919-923, 1996 to state "random integration of transgenes results in a concatameric array of multiple copies, whereas single copy integrations are unusual". Thus, it is clear from the cited art that, the method of a gene knockdown resulting from method routinely used for RNAi effect for nonhuman vertebrate for stably integrating by homologous recombination an expression vector in polymerase II dependent locus is considered unpredictable and an artisan would have to undue experimentation to achieve sustained higher expression of shRNA.

In response, to applicants argument that claims should be not limited to any specific phenotype, it is emphasized that none of the method claim embrace any phenotype of inhibiting or lowering the expression of transgene, therefore given broadest reasonable interpretation instant method does not even require inhibition of transgene. Therefore, as recited instant method embraces methods that do not result in any specific phenotype associated with stable integration of vector comprising shRNA. The art of record teaches that attenuation /inhibition of the targeted gene, the degree of attenuation, length of the time attenuation and induction of interferon pathways are also other factors that not predictable and are responsible for varying effect of RNAi (Prawitt et al and Caplen et al Gene 2000, vol. 252, 95-105, art of record). It is noted that specification has exemplified a method to stably integrate transgene by homologous recombination in the genome of the nonhuman vertebrate using mouse ES cells. However, claims embrace a method of gene knockdown in any nonhuman vertebrate, however, at the time of filing of this application ES cell technology was generally limited to the mouse system and that only putative ES cells exist for other species. It is noted that the unpredictability of a particular art area may alone provide reasonable doubt as

to the accuracy of the broad statement made in support of enablement of claims. See *Ex parte Singh, 17 USPQ2d 1714 (BPAI 1991)*. It is also well established in case law that the specification must teach those of skill in the art how to make and how to use the invention as broadly claimed. *In re Goodman, 29 USPQ2d at 2013 (Fed. Cir. 1994)*, citing *In re Vaeck, 20 USPQ2d at 1445 (Fed. Cir. 1991)*. An artisan would have to perform undue experimentation to obtain ES cell from different nonhuman vertebbrate and other elements that functions well in that nonhuman vertebrate to stably integrate by homologous recombination and expression vector comprisng shRNA (emphasis added).

New-Claim Rejections-Necessitated by amendments - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 15-17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 15 recitation of the limitation "construct to be integrated into a polymerase III dependent locus" is unclear to the extent independent claim 1 is directed to a method of gene knock down by stably integrating by homologous recombination an expression vector into polymerase II dependent locus. It is not apparent how claims 15-17 would further limit the method of claim 1 by integrating the vector in polymerase III dependent locus. Appropriate correction is required.

Claims 1, 5-6, 8-24, 26-26 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Instant claims are directed to a method for gene knockdown comprising stably integrating by homologous recombination into a polymerase dependent locus an expression vector comprising shRNA construct, but the does not set forth any steps involved in method that would facilitate homologous recombination, it is unclear what method /process applicant is intending to encompass. The omitted steps are: whether homologous recombination is achieved by integrating expression vector in ES cell of the nonhuman vertebrate or by random integration. The claim merely recites a method of gene knock down by administering the a vector by homologous recombination without any active, positive step linking to the preamble indicating whether method actually results in gene knock down phenotype. Claims 2-10, 13, 16-23 directly or indirectly depends on claim 1. Appropriate correction is required.

Withdrawn-Claim Rejections - 35 USC § 102

Claims 27-, 29-30 rejected under 35 U.S.C. 102(b) as being anticipated by Buvoli et al (Mol Cell Biol. 2000; 20(9): 3116-24) is withdrawn in view of amendments to the claim now reciting specific limitation in the construct and non human vertebrate comprising the shRNA construct.

Withdrawn-Claim Rejections - 35 USC § 102

Claims 1-2, 5, 7-11, 13-14, 24, 26-27 and 29 rejected under 35 U.S.C. 102(e) as being anticipated by Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) is withdrawn in view of amendments to the claims.

Maintained-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1, 5-10, 13-24, 26-27 and 29-30 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072) and Soriano et al (US patent 6,461,864, October 8, 2002).

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either *in vivo* or *in vitro*. RNA can be derived from an expression construct (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of firefly or Renilla luciferase (pp22; paragraph 246). Beach et al demonstrates that short hairpins encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) *in vivo*. DNA oligonucleotides encoding 29 nucleotide hairpins corresponding to firefly luciferase were

inserted into a vector containing the U6 promoter. Beach further disclose that one of skill can choose from amongst a range of vectors to either transiently or stably express a short hairpin. Beach et al also disclose non-limiting examples of vectors and strategies to stably express short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). It is noted that Beach et al also disclose that promoters/enhancers that may be used to control the expression of the targeted gene in vivo may include cytomegalovirus (CMV) promoter (see para. 147). Beach et al teach and claim a non-human transgenic mammal having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Beach et al also demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6). However, Beach et al do not explicitly teach how an expression vector integrates through homologous recombination at polymerase II dependent locus.

Prior to instant invention, Bronson describes transgenic mice made by pro nuclear injection of DNA as an effective method of achieving expression of exogenous DNA sequences for many purposes, including over expression, mutant analysis, promoter analysis (see page 9067, column 1, para 1). Bronson also describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. It is noted that Bronson provided motivation of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT. He discloses many advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible with a desired developmental and tissue-specific expression. Bronson also taught homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome (see page figure 2 and page 9068, column 2, para 3). However, Bronson et al do not teach expressing shRNA in a specific locus.

Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous

DNA segment in Rosa 26 locus (abstract and claim 1). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54. Soriano also discloses a schematic of G2BP gene showing the retroviral promoter trap insertion site and a cassette comprising the ROSAbgeo retroviral insert. Soriano also shows structural motifs associated with RNA binding protein SA, splice acceptor, LTR, long terminal repeat; SH; SH3 domain binding sequence (column 4, figure 2). Thus, Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al do not teach a method of using shRNA construct in rosa26 locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method the construct disclosed by Beach to include the shRNA construct into a specific locus by homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided motivation by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Soriano had already disclosed the methods and vector constructs for the production of non-human transgenic animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus. The person of ordinary skill in the art would have been motivated to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as ROSA26 or HPRT by homologous recombination as discussed by Bronson and Soriano, as it would have suppressed the expression of transgene for sustained period.

One who would practiced the invention would have had reasonable expectation of success because Beach et al had already described a method for gene knockdown in

a mice by transiently as well as stably expressing shRNA construct and it would have only required routine experimentation that were disclosed by Bronson and Soriano before filing of this application. One of ordinary skill in the art would have been motivated to combine the teaching of Beach, Bronson and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific ROSA26/HPRT locus would have provided stable and sustained inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 5-24, 26-27 and 29-30 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072); Soriano et al (US patent 6,461,864, October 8, 2002) and Ohkawa et al (Hum Gene Ther. 2000; 11 (4): 577-85; IDS).

The combined teachings of Beach, Bronson and Soriano have been discussed above and are relied upon in same manner.

Ohkawa et al teach several constructs composed of the human U6 snRNA promoter and sequences derived from the gene for the tetracycline operator of a prokaryotic tetracycline resistance transposon (abstract). Ohkawa also disclose that expression of the promoter of the human gene for U6 snRNA that contains tet O sequences between the PSE (Figure. 1 and 2) and a TATA box could be be efficiently repressed in cells with the Tet repressor and that this repression can be reversed by tetracycline. Ohkawa et al used this expression system to control the function of an antisense RNA for a fusion gene composed of genes for epidermal growth factor receptor (EGFR) and green fluorescent protein (GFP) and expression of this chimeric gene could be efficiently and rapidly inhibited by tetracycline. However Ohkawa et al do not teach a method to gene knockdown in a nonhuman vertebrate.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the construct and method disclosed by Beach to include inducible promoters for shRNA construct wherein operator sequence consist tet as disclosed by Ohkawa. The skilled artisan would be further motivated to include this construct in a specific locus by homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome wherein transgene could be regulated by tetracyline. Ohkawa provided the provided motivation by showing that tet based system could control the expression of transgene, while Bronson emphasized the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Soriano had already disclosed the methods and vector constructs for the production of non-human transgenic animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus. The person of ordinary skill in the artn would have been motivated to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as ROSA26 or HPRT by homologous recombination as discussed by Bronson and Soriano as it would have suppressed the expression of transgene for sustained period.

One who would practiced the invention would have had reasonable expectation of success because Beach et al had already described a method for gene knockdown in a mice by transiently or stably expressing shRNA construct and it would have only required routine experimentation that were disclosed by Ohkawa, Bronson and Soriano, before filing of this application to a method for gene knockdown in a nonhuman vertebrate as recited in the instant application. One of ordinary skill in art would have been motivated to combine the teaching of Beach, Bronson, Ohkawa and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a tet based inducible promoter into a specific ROSA26/HPRT locus would have provided stable and sustained regulated inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Response to Arguments

Beach et al; Bronson et al and Soriano et al

Applicant's arguments filed January 26, 2007have been fully considered but they are not persuasive. Applicants concede that Beach demonstrates that a lucifemse specific shRNA under the control of the U6 promoter can mediate widespread gene silencing in cultured cell lines (referred as "in vivo '" in this document). However, the document lacks any information about shRNA mediated RNAi in a multicellular organism (See page 11). Applicants argue that thus Beach et al do not teach or suggest gene silencing via shRNA expression in transgenic animal. Applicants also assert that Beach et al did not further analyze transgenic cell lines in respect to the integration site. Therefore, it was not obvious to a person skilled in the art that a polymerase III dependent promoter could exert sufficient activity when integrated into a polymerase II dependent locus. Applicants further state that usually, random integration of transgenes results in a concatameric array of multiple copies, whereas single copy integrations are unusual (Martin & Whitelaw, BioAssays 18. 919-923 (1996)). Applicants conclude that it was questionable whether a single copy of a siRNA expression vector integrated into the genome would result in sufficiently high levels of siRNA required for RNAi-mediated gene inhibition in multiple organs of the living organism. Further applicants describe to overcome problems of random transgenesis Bronson applied homologous recombination to introduce a single transgene copy into the HPRT locus. Applicants describes variable expression pattern and other problems of HPRT locus (Hatada et al., J. Biol., Chem., 274(2). 948-55, 1999 and Guillot et al., Physiol. Genomics, Mar. 13, (2)." 77-83 (2000). Applicants conclude that therefore, the combination of Beach and Bronson did not provide motivation of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT for

ubiquitous expression. Applicants then state that Soriano describes a method for the production of transgenic animals, which ubiquitously express a heterologous gene inserted into the Rosa26 locus through homologous recombination. In this configuration, the endogenous rosa26 promoter drives transgene expression via a splice acceptor sequence. Applicants assert that, Soriano did not invest activity of exogenous promoters when stably inserted into rosa26. Applicants concludes that the combination of Beach, Bronson and Soriano did not provide motivation to insert transgenes under the control of an exogenous polymerase III-dependent promoter into rosa26 to achieve ubiquitous expression.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). It is noted that applicants concede that Beach demonstrates that a luciferase specific shRNA under the control of the U6 promoter can mediate widespread gene silencing in cultured cell lines. Applicants assert, the document lacks any information about shRNA mediated RNAi in a multicellular organism (See page 11). Examiner would agree that Beach et al did not exemplify any transgenic animal showing reduced expression of any transgene *in vivo*. However, contrary to applicants arguments Beach et al clearly contemplated a non-human transgenic mammal having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Furthermore, Beach et al not only disclosed vectors and strategies to stably express short dsRNAs using U6 and H1 promoters, but also taught promoters/enhancers that may be used to control the expression of the targeted gene in vivo may include cytomegalovirus (CMV) promoter (supra). Examiner would agree that Beach contemplated random transgenesis (supra) for suppressing the expression of transgene. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., siRNA expression vector integrated into the genome would result in sufficiently high levels of siRNA required) are not recited in the

rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). It is noted that applicants have individually analyzed the references. In the instant case, Bronson describes disadvantages of transgenic mice made by pro nuclear injection of DNA including random integration and unpredictable copy numbers. It is emphasized that Bronson references is included to demonstrate that advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible (supra) and not necessarily at HPRT locus as argued by the applicants. In response to Applicants argument that, Soriano did not invest activity of exogenous promoters when stably inserted into rosa26. It is emphasized that applicants are arguing to a feature (activity of a promoter) that is not required in the rejected claims. Contrary to applicants argument none of the method claims require any specific activity. Furthermore, Soriano et al only teach methods and vector constructs for the production of genetically engineered non-human animals, by targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26 locus sequence (column 3, lines 51-54. Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. Although, Soriano did not exemplify a method that uses polymerase II dependent promoter, however, Soriano contemplated operation of a promoter may vary depending on its location in the genome. He clearly stated that a regulated promoter may operate differently from how it does in its normal location, e.g., it may become fully or partially constitutive. In addition, Soriano et al also contemplated inducible promoters. Thus, it is apparent that contrary to applicants arguments, it would have been obvious for one of ordinary skill in the art at the time of invention to modify the method the construct disclosed by Beach to include the shRNA construct into a specific locus by homologous

recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome at polymerase II dependent locus as taught by Soriano. Bronson provided motivation by emphasizing the problems associated with random transgenes and advantages of using a chosen site for a single copy of a transgene (see page 9072, col. 1, last paragraph). Furthermore, Soriano had already disclosed the methods and vector constructs for the production of non-human transgenic animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus. The person of ordinary skill in the art would have had reasonable expectation of success for a method of reducing gene expression by stably integrated expression vector comprising an shRNA into a specific locus such as ROSA26 or HPRT as discussed by Bronson and Soriano. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Beach et al; Bronson et al; Soriano et al and Ohkawa et al

Applicants argue that Okhawa does not overcome the above-noted deficiencies in the combination of Beach, Brortson and Soriano.

In response, it is emphasized that the reference of Okhawa et al is included to demonstrate that use of inducible promoter containing operator sequence such as tet was routine in the art. It would have been prima facie obvious to one of ordinary skill in the art to include tet based system to control the expression of transgene by combining the teaching of Beach, Brortson and Soriano as summarized in preceding section

Conclusion

No Claims allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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